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Significance of Placental Damage in Vertical Transmission of Human Immunodeficiency Virus

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The significance of physical breaches of the trophoblastic layer of the placenta in transmission of HIV from mother to infant was evaluated in 17 HIV-infected pregnant women. Samples of peripheral blood were obtained from the women during pregnancy and at delivery, at which time a small piece of placental tissue was obtained from a random site and immediately placed into fixative. Blood samples were obtained from infants at or shortly after birth and thereafter at approximately 3-month intervals, until the age of 18 months, in order to determine their HIV infection status. HIV RNA and p24 antigen were quantified in maternal plasma and CD4 cells enumerated. Paediatric diagnosis was conducted using polymerase chain reaction, virus isolation, detection of p24 antigen, and measurement of class-specific antibodies. Placental damage was quantified and evaluated using transmission electron microscopy. Maternal viral load was low, with a mean RNA copy number of 8,237 per millilitre of plasma (range 230–37,233 copies/ml). Only two women were p24-antigenaemic, and CD4 numbers ranged from 0.09 to 2.8×10^9/l. There was evidence of breaks in the trophoblastic surface to the depth of the basement membrane in all 17 placentas, and perivillous fibrinoid deposits were also observed to a varying degree in all samples. However, none of the 13 infants available for follow-up had evidence of infection with HIV. Superficial damage to the trophoblastic surface of the placenta, with exposure of the basement membrane and potential exposure of CD4-expressing cells, does not appear to be a significant factor in the transmission of HIV from mother to infant during pregnancy.
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KEY WORDS: placenta, human immunodeficiency virus, vertical transmission

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INTRODUCTION

Transmission rates of human immunodeficiency virus (HIV) from mother to infant have been reported to range from 14% to 40%. Studies have demonstrated that transmission may occur in utero, during delivery, or postnatally as a result of breast-feeding, although the relative proportion and efficiency of transmission at these times is unclear. Although accumulated clinical, serological, and virological data suggest that 50–80% of transmissions may occur at or during delivery [Mofenson, 1995], more accurate information is required on the timing and mechanisms involved as this is likely to have a significant effect on the success of intervention strategies to prevent materno-foetal transmission.

The route by which antepartum infection occurs, and potential mechanisms from the time of fertilization onwards, have recently been reviewed [Schwartz and Nahmias, 1991; Douglas and King, 1992; Borkowsky and Krasinski, 1992; Ebbesen et al., 1994]. However, despite the placenta providing a potential barrier between the maternal and foetal circulations, only limited attention has been focused on its possible role in the transmission of HIV.

The human placenta is of the villous haemochorial type and consists of a vast array of foetal villi, which are bathed directly by circulating maternal blood [Kaufmann and Burton, 1994]. At the end of pregnancy, the villi present a surface area of 10–14 m^2 and so permit extensive and intimate contact between foetal tissues and maternal blood. The outermost covering of the villi is the syncytiotrophoblast, which is a continuous multinucleated epithelium generated from and maintained by an underlying population of mononuclear cytotrophoblast cells. This trophoblastic layer is supported by a basement membrane, which separates it from the mesenchymal cells of the villous core. Within the core are

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TABLE I. Clinical and Virological Details of the Mother–Infant Pairs

Subject no.	Clinical status	Mean CD4 no. (10^9/l)	Zidovudine treatment	p24 antigenaemia	Mean RNA copy (no./ml plasma)	Delivery details*	% Placental damage	HIV status of infant
1	Asymp	ND	Yes	Neg	806	Vaginal, 37 wk	4.3	Uninfected
2	Asymp	0.45	No	Neg	1,922	Vaginal, 40 wk, ARM	8.2	Uninfected
3	Asymp	0.40	No	Neg	230	Vaginal, 41 wk, ARM	1.8	Uninfected
4	Asymp	0.68	No	Neg	2,580	Caesarian, 42 wk	0.9	Uninfected
5	Asymp	0.37	No	Neg	2,273	Vaginal, ARM	2.2	Uninfected
6	Asymp	0.23	No	Neg	8,833	Vaginal	17.8	Uninfected
7	Asymp	0.10	No	Pos (43 pg/ml)	36,750	Vaginal	16.9[a]	Uninfected
8	Asymp	0.18	No	Neg	37,233	Caesarian, 40 wk	14.3	Uninfected
9	Symp	0.09	No	Neg	1,620	Vaginal	0.8	Uninfected
10	Asymp	2.8	No	Neg	6,730	Vaginal, 41 wk	0.3[b]	Uninfected
11	Asymp	2.3	No	Neg	1,193	Vaginal, ARM, forceps, Epis	8.2	Uninfected
12	Asymp	2.2	Yes	Neg	270	Vaginal, 38 wk	2.2	Uninfected
13	Asymp	ND	No	Pos (74 pg/ml)	17,000	Caesarian	6.4[c]	Indeterminate
14	Asymp	0.75	No	Neg	2,400	Vaginal, ARM	3.0	Unknown
15	Asymp	1.1	Yes	Neg	4,933	Vaginal, 39 wk	5.0	Indeterminate
16	Asymp	0.85	Yes	Neg	2,266	Vaginal, 42 wk, ARM	5.9	Indeterminate
17	Asymp	2.5	No	Neg	13,000	Vaginal, Epis	3.7[d]	Unknown

*Epis, Episiotomy; ND, not done; ARM, artificial rupture of membrane.
[a]Particles observed in trophoblastic basement membrane.
[b]Aggregation of platelets and neutrophils in intervillous space.
[c]Particles observed budding from leucocyte in placental capillary.
[d]Particles observed budding from cytotrophoblast cell.

the foetal capillaries and a significant number of macrophages, often referred to as Hofbauer cells, some of which express the CD4 molecule [Goldstein et al., 1988; Nakamura and Ohta, 1990]. In contrast, CD4 is not expressed on the syncytiotrophoblastic surface [Douglas and King, 1992]. In view of this and its lack of intercellular junctions, the outer covering of the villi might be expected to present a significant barrier against placental infection by HIV. However, breaks in the continuity of the syncytiotrophoblastic surface can occur from early pregnancy through to term [Kline, 1951; Burton, 1987, 1994], and these defects act as foci for perivillous deposition of fibrinoid [Fox, 1978; Benirschke and Kaufmann, 1990]. Such damage could theoretically provide a potential mechanism for infection of the placenta and subsequent transmission of virus to the foetus in utero.

The aim of this study was therefore to evaluate whether physical breaches of the trophoblastic surface play a role in the vertical transmission of HIV.

MATERIALS AND METHODS

Study Population

Samples were collected from 17 HIV-infected women attending antenatal clinics in London and Dundee. These women were participants in a larger multicentre study evaluating factors influencing materno-foetal transmission of HIV. Local ethical approval for the study was obtained, as was informed consent from all women. The clinical and virological details of these women are given in Table I. Four of the women were treated with Zidovudine during pregnancy; intraveneous infusion during labour was not administered. All four infants were treated with Zidovudine from birth. Three of the deliveries were caesarian sections (two elective, one emergency), and none of the women breast-fed their infant.

Specimens

Two to three blood samples were collected from the women during pregnancy and another at or shortly after delivery. Immediately after delivery a block of placental tissue was removed from a random site and placed in 3% glutaraldehyde in 0.1 M Pipes buffer. Blood was collected from the majority of infants 24–48 hr after birth and thereafter at approximately 3-month intervals to determine whether they had been infected with HIV.

Virological and Immunological Studies

Viral RNA was quantified in sequential maternal plasma samples using the NASBA HIV-1 RNA QT assay (Organon Teknika, Durham, NC) and levels expressed as a copy number per millilitre of plasma. Levels of p24 antigen were quantified using an enzyme immunoassay (EIA) employing acid dissociation (NEN Du Pont, Stevenage, UK). CD4 cells were enumerated using flow cytometry. Paediatric diagnosis was made using criteria we have previously discussed [McClure et al., 1995]. The following assays were used: polymerase chain reaction (PCR), virus isolation, acid-dissociated p24 antigen detection, and measurement of HIV-specific IgG, IgA, and IgM (class-specific antibody assays were kindly conducted by Dr John Parry, PHLS, Colindale, UK).

Tissue Samples

Placental tissue was diced into small cubes, and four pieces per placenta were randomly selected for further processing. These were postfixed in 1% osmium tetroxide, dehydrated through ascending concentrations of alcohol, and embedded in Spurr resin. Sections 1 μm thick were stained with methylene blue for light microscopy. Thin sections were stained with uranyl acetate and reviewed in a Philips CM 100 electron microscope.

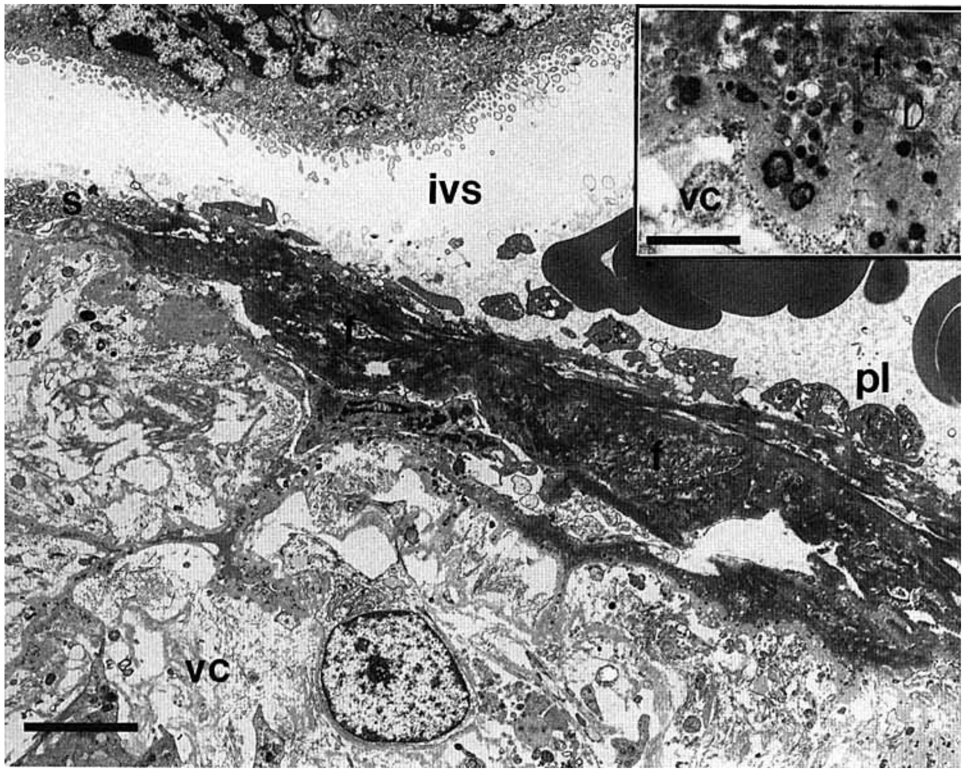

Fig. 1. Subject 3: A low-power transmission electron micrograph showing an area of the villous surface which is devoid of a trophoblastic covering. The normal covering of syncytiotrophoblast (s) visible at the left-hand margin is replaced by a mass of fibrinoid (f). Maternal platelets (pl) circulating in the intervillous space (ivs) adhere to the surface of the fibrinoid. Degenerating cells are frequently observed entrapped in the fibrinoid, and cell debris accumulates close to the trophoblastic basement membrane (inset), which separates the trophoblast from the villous core (vc). Scale bar = 5 μm (inset = 1 μm).

Morphometry

In order to obtain an estimate of the extent of villous damage in each of the placentas, the 1 μm sections were viewed under a light microscope. Fields were selected in a systematic fashion with a random start point [Mayhew and Burton, 1988], and villous profiles were overlain with a quadratic test grid. The number of points falling on perivillous fibrinoid were counted and expressed as a percentage of the total number of points falling on villous tissue. This provided an estimate of the volume fraction of the villous tissue occupied by fibrinoid for each placenta.

RESULTS

Virological and Immunological Studies

Viral RNA was detected in all maternal plasma samples. RNA levels were consistent in sequential samples from individual women, with less than a 1 log difference in copy number between samples. There was therefore little fluctuation in viral load during pregnancy. The mean RNA copy number per millilitre of plasma is given in Table I. RNA levels ranged from 230 to 37,233 copies/ml (mean 8,237 copies/ml), which is consistent with levels we have previously detected among nonpregnant asymptomatic individuals (unpublished data). Three of the women treated with Zidovudine (subjects 1, 12, and 16) were amongst those with the lowest viral load. Only two of the 17 women (11.7%) were p24-antigenaemic and both were amongst those with high plasma RNA levels. One of these women (subject 7) had p24 antigen in three plasma samples collected during pregnancy and in the delivery sample; the mean concentration (43 pg/ml) is given in Table I. Only one sample, collected at delivery, was available from the other woman (subject 13) who was antigenaemic. CD4 numbers during pregnancy were fairly consistent in sequential samples from individual women, and mean numbers are given in Table I (range 0.09–2.8 × 10^9/l).

Infants were classified into three groups based on HIV infection status (Table I). Uninfected status was defined on the basis of negative PCR, virus isolation, and p24 antigen detection in sequential samples and seroreversion by the age of 18 months. Indeterminate infection status was defined by negative PCR, virus isolation, and p24 antigen detection in sequential samples from infants younger than 18 months in whom seroreversion had not yet occurred. Unknown infection status refers to infants lost to follow-up. Twelve of the 17 infants were uninfected and three (subject 13, 15, and 16) of indeterminate status. One of these three children (subject 13), currently aged 13 months, has had consistently negative PCR, virus isolation, and p24 antigen results. Declining levels of HIV antibodies have also been detected in sequential samples, and only a weak antibody response was detected

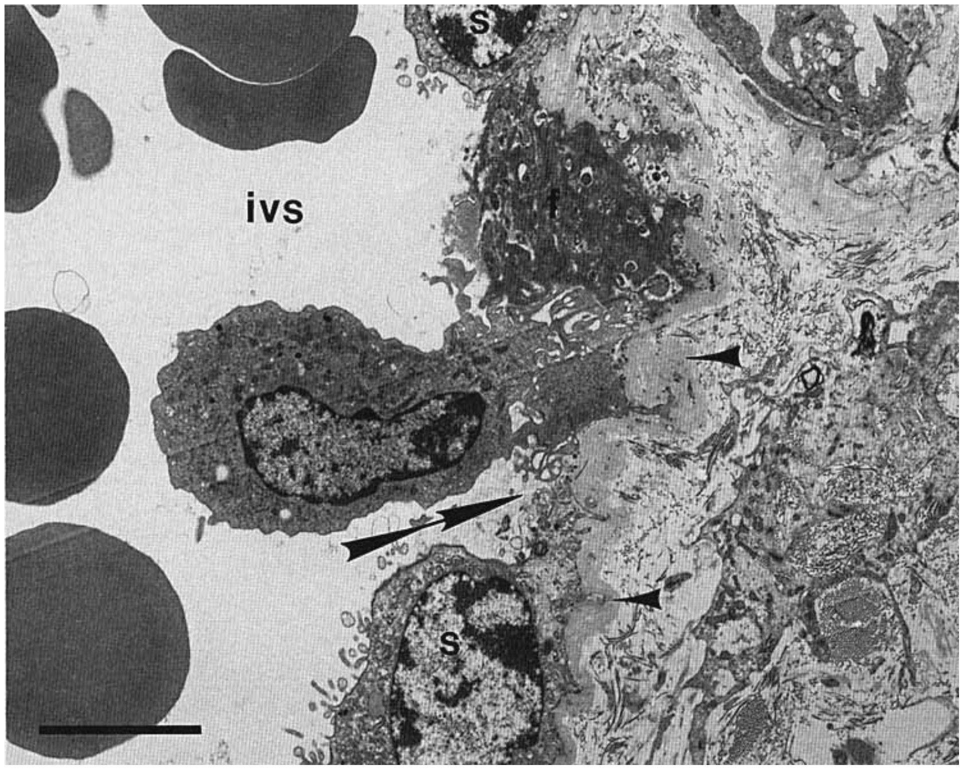

Fig. 2. Subject 4: An example of a gap in the syncytiotrophoblast layer (s) which is not completely plugged by fibrinoid (f). The trophoblastic basement membrane (arrowheads) is exposed directly to the contents of the intervillous space (ivs).

in the most recent sample collected at the age of 13 months; these results therefore suggest that this child is uninfected. Two infants (subjects 15 and 16), currently aged 20 and 18 months, were PCR- and virus isolation-negative 24 hr after birth, but further laboratory tests have been refused by the parents. These two children are, however, asymptomatic. Two children (subject 14 and 17) have been lost to follow-up and are therefore of unknown infection status. However, both children were negative by PCR and virus isolation at birth and one infant (subject 14) was also negative when tested at the age of 4 months.

Placental Studies

There was no evidence of any acute or chronic inflammatory response within the villi examined, and in only one case (subject 10) was there an accumulation of maternal neutrophils and platelets within the intervillous space. Placental damage, as determined by the percentage of perivillous fibrinoid deposits, was observed to a varying degree in all placentas (Table I). Deposits fell into two categories, morphologically: those which were covered by a thin layer of syncytiotrophoblast and those which were not. The former were larger and more extensive than the latter, suggesting that they may have been of longer standing. Both types of deposit were seen in all placentas.

Electron microscopy demonstrated platelets adhering to the villous surface wherever the covering of syncytiotrophoblast was deficient. Most breaks in the trophoblast surface were filled with a dense meshwork of fibrin fibres, which often made extensive contact with the basement membrane (Fig. 1, subject 3). The basement membrane was noticeably thicker in these areas than under the healthy syncytiotrophoblast. Numerous vesicles were present within the fibrinoid, along with fragments of activated platelets. Frequently these vesicles were in close proximity to the basement membrane (Fig. 1 inset). In some of the larger deposits what appeared morphologically to be maternal neutrophils were trapped within the superficial layers.

Not all defects in the trophoblastic surface were completely filled with fibrinoid. In Figure 2 (subject 4), a small defect is illustrated in which a deposit only partially filled the gap. In the centre of the defect were cells which protruded into the intervillous space but which also made intimate contact with the basement membrane. The morphological appearances and position of these cells indicate that they were most likely maternal neutrophils which were infiltrating the trophoblastic defect. However, in the absence of confirmatory immunocytochemistry, the possibility that they were cytotrophoblast cells migrating out from the wound cannot be excluded. Between these cells and the margins of the syncytiotrophoblast, the trophoblastic basement membrane was freely exposed to the contents of the intervillous space.

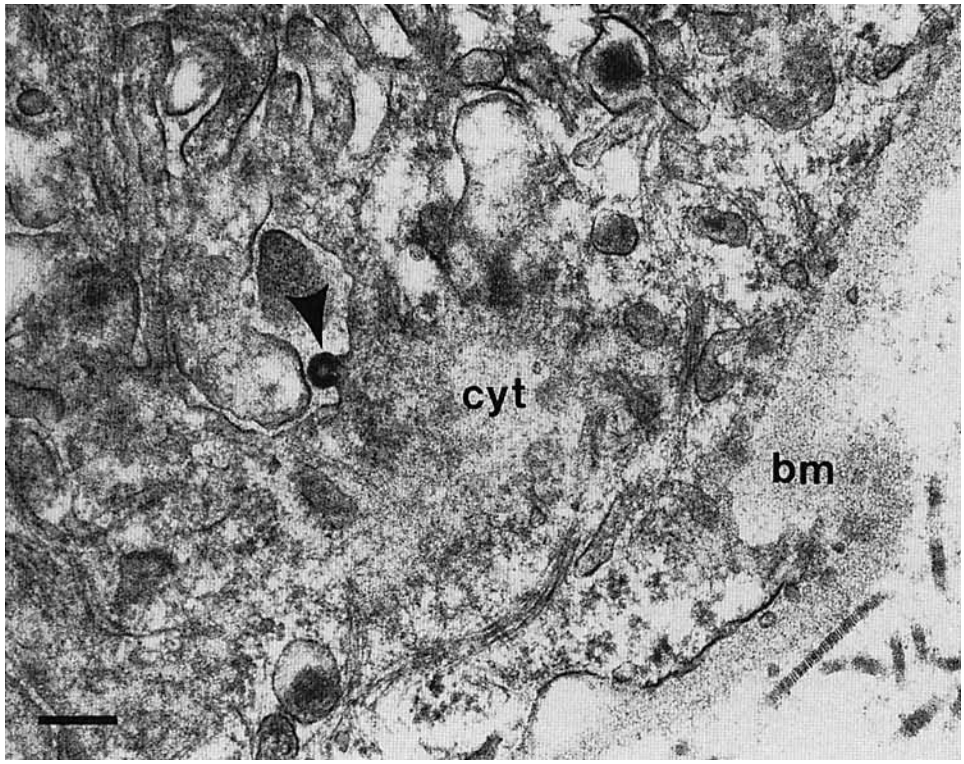

Fig. 3. Subject 17: An example of a viral particle (arrowhead) budding from a cytotrophoblast cell (cyt) lying on the trophoblast basement membrane (bm). The appearances are consistent with either an endogeneous type C retrovirus or an HIV particle. Scale bar = 200 nm.

Despite all the women being infected with HIV, there was little evidence of viral infection in any of the placentas. The appearance of a budding viral particle from a cytotrophoblast cell was observed in only one placenta (Fig. 3, subject 17), and the appearance was consistent with either an endogeneous Type C retrovirus or an HIV particle, although the former are more usually observed in association with the syncytiotrophoblast. Free particles consistent in size with HIV were seen within the trophoblastic basement membrane in one other case (subject 7). Macrophages within the villous core were present in all placentas, often in close proximity to the trophoblastic basement membrane, but there was no evidence of HIV by electron microscopy. Budding of viral particles from the surface of leucocytes within the foetal capillaries was observed in one case (subject 13, Fig. 4), and ranged from the aggregation of an electron-dense mass immediately below the plasma membrane to the formation of almost complete spherical particles. The latter were 100 nm in diameter and, although their morphology was consistent with that of HIV, this was not confirmed immunocytochemically. Virological data suggest that this infant is unlikely to be infected with HIV.

DISCUSSION

This study is important because it demonstrates that the trophoblastic surface can be breached without foetal infection occurring. Considerations of whether the trophoblast can be infected with HIV and how the virus might traverse it are therefore unlikely to be relevant in terms of transmission of HIV from mother to infant. No matter how the virus reaches the basal surface of the trophoblast, it still has to pass through the basement membrane and negotiate the stromal macrophages. Although the budding particle shown in Figure 3 is consistent morphologically with HIV, this could also be an endogenous retrovirus of the type illustrated by Lyden et al. [1994]. The latter are usually seen on the basal surface of the syncytiotrophoblast, whereas that shown in Figure 3 is clearly arising from a cytotrophoblast cell. This child (subject 17) has been lost to follow-up, and it is therefore unknown whether transmission of HIV occurred. Evidence for the involvement of placental macrophages in vertical transmission is conflicting [Lewis et al., 1990; Martin et al., 1992; Peuchmaur et al., 1991], but these cells can be infected with monocyte-tropic strains of HIV-1 in vitro [Melendez-Guerrero et al., 1994]. Although macrophages were identified within the villous core of all the placentas examined, none appeared morphologically to be harbouring HIV.

The morphometric data presented here cannot be taken as accurate estimates of the amount of perivillous fibrinoid deposited in the placentas as the entire organs were not available to us for exhaustive sampling [Mayhew and Burton, 1988]. Despite breaks in the trophoblastic surface being a universal feature of the placentas studied, there was no evidence that this is a signif-

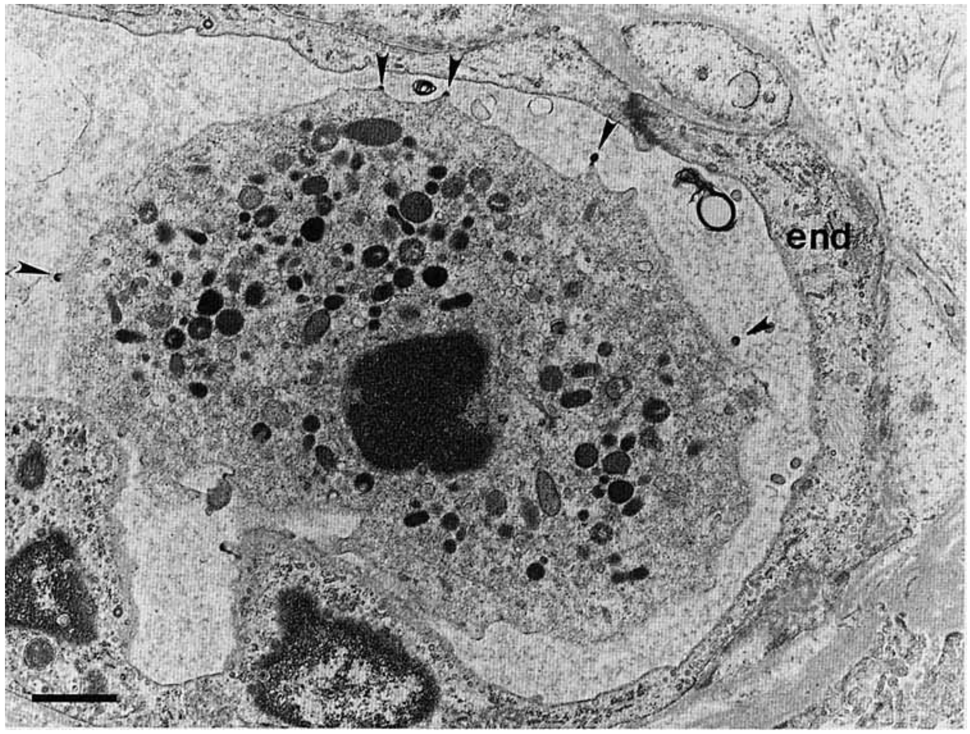

Fig. 4. Subject 13: A leucocyte within a foetal capillary in the placenta demonstrating viral particles budding from the surface (arrowheads). The endothelium (end) appeared normal. Scale bar = 1 μm.

icant factor in mother to infant transmission. A similar incidence of fibrinoid necrosis has been reported in occasional scattered villi in terms placentas from normal uncomplicated pregnancies [Fox, 1968]. It is unlikely, however, that these physical defects represent an important portal of entry for the virus as 13 of the 17 infants were not infected with HIV, with the remaining four being of indeterminate or unknown status.

Superficial breaks in the trophoblastic surface most probably occur throughout gestation, and one cause that has been well documented is the rupture of points of syncytial fusion between neighbouring villi [Burton, 1986, 1994]. Other possible causes include ischaemic episodes and shearing forces induced by physical movements of the foetus [Eden, 1987]. However, such breaks are likely to be covered rapidly through platelet activation and so may be of no significance in transmission of HIV due to the fibrinoid plug providing an effective seal. This could be questioned, however, on the basis of the morphological evidence presented here, for breaches of the trophoblastic epithelium such as the one illustrated in Figure 2 clearly expose the basement membrane to the contents of the intervillous space. We have no means of assessing the duration of this exposure, however, and how soon platelet activation would follow. Nonetheless, vesicles and particles released from degenerating trophoblast and possibly from entrapped maternal leucocytes can reach the basement membrane. Horseradish peroxidase (48,000 D; 3.0 nm molecular radius) perfused into the intervillous space in vitro can do the same [Edwards et al., 1993]. It is unclear, however, whether HIV has the ability to penetrate the fibrinoid matrix. In addition, the lesions described here are all superficial to the trophoblastic basement membrane. This remains intact and is often locally thickened under sites of perivillous fibrinoid. The membrane is composed largely of type IV collagen, laminin, and fibronectin and could therefore present a significant barrier to the virus.

Given the transient nature of these defects in the trophoblastic surface, which are likely to occur throughout gestation, another factor which must be taken into account when assessing their significance for transmission of HIV is the maternal viral load during pregnancy. The level of maternal viraemia coincident with the damage may determine whether or not placental infection and subsequent transmission to the foetus occurs. Only one (subject 9) of the 17 women in this study had symptomatic disease, although her plasma RNA copy number was not particularly high. RNA copy numbers in the remaining women were consistent with asymptomatic disease. We have previously demonstrated that plasma RNA levels are at least 100-fold higher in symptomatic than asymptomatic individuals (unpublished data), and in a pregnant woman this could be critical in terms of whether or not transmission occurs, whether this be in utero or during delivery following exposure of the infant to infected maternal blood and genital secretions. It is notable that the placenta in which free particles were observed within the basement membrane came from a mother with one of the highest RNA copy numbers who

was also p24-antigenaemic (subject 7, Table I) and recent data [Fang et al., 1995; Zoller et al., 1996] have suggested that levels of viral RNA in maternal blood may predict whether or not transmission occurs.

Minor trophoblastic damage may be of little significance to vertical transmission for several reasons. The intact trophoblastic basement membrane may provide an effective barrier against the virus. Alternatively, viral particles may be able to pass through the basement membrane but are then sequestered in the macrophage population within the stromal core. A third possibility is that virus reaches the foetal circulation but is subsequently cleared. HIV-specific cytotoxic T cells may be important in this respect, and such responses have been detected in newborns of infected mothers who, although exposed to the virus, have no evidence of infection [Rowland-Jones et al., 1993; De Maria et al., 1994]. Regardless of the mechanisms involved, however, an important factor influencing whether or not infection occurs may be the viral load to which the foetus is exposed.

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